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Full Length Article

Nitrogen Nutrition Diagnosis Based on Critical Nitrogen Model and SPAD Value of Different Leaf Positions in Greenhouse Tomato

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Abstract

This study investigated the applicability of the critical nitrogen dilution curve model of greenhouses to propose a method for quickly and accurately diagnosing nitrogen status of tomato using chlorophyll meters. Water and nitrogen experiments were conducted from 2016 to 2018. The water treatments included were full irrigation during the whole growth period and reducing irrigation of 50% during the seedling stage, seedling and flowering stages, during the whole growth period. The nitrogen application rates were 0, 150 and 300 kg/hm^2. The applicability of the critical nitrogen concentration dilution model in the greenhouse environment was verified. Based on the experimental data, the correlation between SPAD value and nitrogen nutrition index (*NNI*) of tomato leaves at different leaf positions was analyzed. The results showed that nitrogen nutrition dilution model is suitable for different water treatment of greenhouse tomatoes and significant linear relationship exists between SPAD values of the middle tomato leaf and *NNI*. The SPAD value of the middle leaf can diagnose the nitrogen status of tomato and when *NNI* is 1, it can be used as an indicator for proper nitrogen application. Therefore, this study can provide a good theoretical reference for real-time nitrogen nutrition diagnosis and optimized nitrogen management of greenhouse tomatoes. © 2020 Friends Science Publishers

Keywords: Greenhouse; Tomato; Water treatments; Critical nitrogen concentration; Nitrogen nutrition index

Introduction

Greenhouse vegetable cultivation has developed rapidly in northwestern China. Tomatoes, as the main type of greenhouse vegetables, have a short growth period and account for a large proportion of off-season vegetables. Traditional fertilization is used for water and fertilizer management. The phenomenon of excessive application of nitrogen fertilizer is common, and can not only reduce the yield of vegetables, but also lead to the accumulation of nitrate in soil and cause secondary salinization of the soil (Guo *et al.* 2004; Gao *et al.* 2008). The critical nitrogen concentration is defined as the minimum nitrogen concentration when the maximum biomass is obtained during a certain growth stage (Ziadi *et al.* 2008). Therefore, it is clear that the critical nitrogen concentration in different growth stages of tomato dry matter formation can diagnose the nitrogen nutrition status of the plants, and form the basis for the rational application of nitrogen fertilizer in various growth stages of tomato.

In recent years, many studies have conducted research on critical nitrogen concentration dilution models for different crops, mainly cotton (Wang *et al.* 2012), wheat (Jørgen and Olesen 2002; Qiang *et al.* 2015), tomato (Tei *et al.* 2002; Wang *et al.* 2013; Yang *et al.* 2015), sorghum (Oosterom and Carberry 2001), corn (Li *et al.* 2015; Qiang *et al.* 2015) and other crops. Studies have shown that the critical nitrogen concentration dilution curve model can better describe the relationship between aboveground biomass and nitrogen concentration, but due to different factors such as test site, crops and experimental treatments, the nitrogen concentration dilution model parameters are variable. There are also great differences between critical nitrogen concentration dilution model parameters (parameters a and b), so the model parameters need to be optimized according to the actual situation. According to the critical nitrogen concentration dilution curve, Lemaire *et al.* (2008) defined the nitrogen nutrition index (*NNI*), which is the ratio of the measured nitrogen concentration in the upper part to the critical nitrogen concentration. When *NNI*=1, it indicates that the nitrogen nutrition in the crop is suitable, while *NNI*>1 indicates nitrogen nutrient excess, and *NNI*<1 indicates nitrogen deficiency. The method for diagnosing *NNI* is a traditional nitrogen diagnostic technique. This

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method is accurate and reliable, but requires a large sample size and the destruction of plant samples. This technique is complicated, time-consuming, and cannot be accomplished in real-time. These factors have limited the popularization and application of this method in practice (Lemaire *et al.* 1997), and modern instrumental nitrogen diagnostic technologies, such as image and computer vision diagnosis, or SPAD instrument diagnosis, can conveniently and non-destructively determine crop nitrogen. This visual technology is able to determine the nitrogen nutrition of the crop canopy by using an image calculation tool to calculate the nitrogen nutrition of the leaf by the image acquisition (Jia *et al.* 2009), but it is inconvenient to carry in the field and cannot be used to track the information in real time. Observing defects such as measurement; high-light remote sensing technology provides a detailed division of the spectrum in a certain spectral region, and obtains spectral information for more bands. Compared with multi-band remote sensing, the spectral resolution of hyperspectral remote sensing is much higher.

Studies have shown that the chlorophyll content in rice leaves may be either high or low. There is a correlation between the spectral features and the nitrogen nutrient status of the plant that can be detected by spectroscopy (Madeire *et al.* 2000). However, there are many problems influencing the data acquisition, and the cost of the instrument is very high. The chlorophyll meter (SPAD meter) is a portable spectrometer with the advantages of being convenient to carry and allowing for real-time observations and measurements. It is based on the difference in absorption of red and near-infrared light by chlorophyll, according to the transmitted light characteristics of the leaf blades. The relative content of chlorophyll in the leaves is expressed, and then the nitrogen nutrition status of the plants can be estimated based on the known relationship between the chlorophyll and nitrogen contents of the leaves (Zhang *et al.* 2003). Errecart *et al.* (2012) also used the leaf SPAD value to quickly simulate crop *NNI* values by establishing a correlation between leaf SPAD values and plant *NNI* values. Yang *et al.* (2014) compared the regression relationship between SPAD values and *NNI* in different leaf positions of rice, and found that the fitting relationship was stable during the interannual period.

Some have speculated that the SPAD value can be used for nitrogen nutrition diagnosis of rice. In this study, in order to improve the applicability of the critical nitrogen concentration dilution curve model in different water treatments of greenhouse tomato in North China and determine the SPAD values of appropriate leaf positions, the *NNI* values of tomato were estimated for nitrogen nutrition diagnosis. The determinations of leaf position and *NNI* based on leaf SPAD values are aimed at providing a theoretical basis for the rational utilization of nitrogen, the diagnosis of nitrogen nutrition, and the optimal management of nitrogen under different watering conditions.

Materials and Methods

Experimental details and treatments

Experimental material: The experiment was carried out in the greenhouse of Hunyuan, Shanxi Province from 2016 to 2018 (39°42′ N, 113°41′ E). The greenhouse is a non-heated with natural ventilation comprising a steel frame structure, covered with plastic film, facing the north-south direction (length × width × height is 55 m × 6.5 m × 4.6 m), and 1 m wide ventilation is provided at the top and bottom of the greenhouse. The port is equipped with a manual opening and closing device. When the temperature in the greenhouse is >35°C or < 10°C, it can be adjusted by opening or closing the vent. Tomatoes in the greenhouse were planted in the north-south direction. The 0~60 cm soil layer of the greenhouse had the following properties: clay (<2 μm) 23%, powder (2~20 μm) 54%, sand (≥20~2 000 μm) 23%, organic matter mass fraction 3.44%, bulk density 1.43 g/cm^3, saturated water content θ_{sat} 0.43 cm^3/cm^3, field water holding capacity θ_{FC} 0.36 cm^3/cm^3, and withering water content θ_{wp} 0.16 cm^3/cm^3.

Treatments: This study design included two factors: irrigation and nitrogen. Four irrigation levels were used: full irrigation in the whole growth stage of tomato (W_1) and reducing irrigation of 50% during the seedling stage (W_2), seedling and flowering stage (W_3), during the whole growth period (W_4). The three nitrogen levels were used: The nitrogen application rates were of 0 (N_0), 150 (N_{150}) and 300 kg/hm^2(N_{300}), and all combinations of water and nitrogen treatments were tested. These combinations were carried out in three replicates incompletely randomly arranged cells. Each cell area was 6.5 m × 2.4 m = 15.6 m^2, and the cells were separated by a plastic film with a depth of 60 cm.

In this study, the sub-membrane furrow irrigation method was adopted. The irrigation period was 15 days after planting, and the irrigation period of each water treatment was about 7 days. The upper irrigation limit (W_1) was set to 90% of the field water holding capacity (Wang *et al.* 2010). Nitrogen fertilizer was applied as urea (46% nitrogen content), and 40% of the base amount was applied before planting. The remaining 60% was dissolved in the water applied at 70, 90 and 110 days after transferring (Days after transferring, DAT).

The growth stage was divided into seedling stage (Transplant to first fruit set), flowering stage (First fruit set to first fruit maturity) and maturity stage (First fruit maturity to uprooting crops after all fruits are harvested). The planting method was a typical ridge-mulching and mulching cultivation mode. The ridge height was 20 cm while the ridge width of 80 cm. The tomato seedlings were planted on both sides of the ridge according to a single hole. The wide row spacing was 80 cm, and the narrow row spacing of 40 cm, plant spacing of 40 cm, and planting density of 4.2 plants/m^2. Equal amounts of phosphate fertilizer 200 kg/hm^2 (in P) and potassium fertilizer 300 kg/hm^2 (in K) were applied before planting. At planting, the planting water was 20 mm, and no water was applied for 14 days after planting to facilitate the emergence of seedlings. After the tomato seedlings emerged, the test was carried out. On the day of planting, the mulch film was 1.2 m wide and 0.005 mm thick along the north-south side of the greenhouse. The tomatoes were harvested once every 2 days after ripening, and other farming management steps were carried out according to the local routine.

Soil moisture contents

The Trime series soil moisture meter (IMKO Corp., Germany) was immersed in the soil at a distance of 20 cm from the plant, and the soil moisture contents of the wide rows, narrow rows and plants in each plot were measured. Each time before and after irrigation, from the surface to 60 cm depth, the measurement was performed once every 15 cm of depth, and the average value was calculated.

Irrigation amount

The irrigation was started from 15 days after planting, and the upper limit of irrigation was 90% of the field water holding capacity (θ_{FC}). The irrigation amount *I* (mm) is given as:

$$I=10(0.9\theta_{FC}-\theta_i)Z_r \qquad \text{(Eq. 1)}$$

Where θ_i is the soil water content before irrigation, cm^3/cm^3; and *Zr* is the planned wet layer depth, cm, set to 60 cm in this study.

Water consumption

Due to the flat terrain in the greenhouse, there is no surface runoff; the local groundwater is buried deep, so the groundwater supply to the tomato can be neglected; the greenhouse can block the entry of rainfall, so the rainfall can be neglected; and according to the self-made permeameter in the greenhouse, measurement of the 60 cm soil layer can be obtained. There is no deep leakage, therefore, the water balance equation can be simplified to:

$$ET_i=（I_i+\Delta W）/I \qquad \text{(Eq. 2)}$$

Where ET_i is the average daily water consumption in *i* days, mm/d; I_i is the amount of irrigation in *i* days, mm; and ΔW is the amount of change in soil water content in *i* days, mm.

Dry Matter and nitrogen contents

Destructive sampling was performed every 20 days after seedling emergence, and three measurements were taken each time. The fresh stem, leaf and fruit quality of the above ground tomato were weighed and sampled, and to calculate the biomass baked at 105°C for 15 min, and dried at 72°C to constant mass.

The dry matter of each treatment was pulverized and sieved. The total nitrogen content of each organ was measured by the H_2SO_4-H_2O_2 digestion method and a Kjeldahl analyzer (FOSS 2300 type) used to calculate the total nitrogen content of the plant. Nitrogen accumulation in each organ (kg/hm^2) = organ nitrogen content (%) × organ biomass (kg/hm^2), and all the organ nitrogen accumulations were added together to obtain the aboveground plant nitrogen accumulation. Plant nitrogen content (%) = plant nitrogen accumulation (kg/hm^2) / plant biomass (kg/hm^2).

SPAD values

Three uniform plants in each treatment were measured by SPAD-502 to obtain the SPAD values of different node leaves (5, 6, 7, 8, 9, 10, 11, and 12 from bottom to top). The data measured in the 5th, 6th and 7th positions were averaged as the lower leaf SPAD value; the data measured in the 8th, 9th and 10th positions were averaged as the middle leaf SPAD value; and the data measured in the 11th and 12th positions were averaged as the upper leaf SPAD value.

Critical nitrogen concentration dilution curve model

According to the equation for the relationship between the critical nitrogen concentration and the aboveground biomass proposed by Lemaire and Salette (1987), the critical nitrogen concentration dilution curve model is:

$$N_c=a\cdot DW^{-b} \qquad \text{(Eq. 3)}$$

Where N_c is the critical nitrogen concentration value, g/100g; *a* is the critical nitrogen concentration of the plant when the aboveground biomass is 10^3 kg/hm^2; *DW* is the maximum biomass of the crop aboveground, 10^3 kg/hm^2; and *b* is a statistical parameter that determines the slope of the dilution curve of the critical nitrogen concentration.

Nitrogen nutrition index (*NNI*)

In order to further clarify the nitrogen nutrition status of crops, Lemaire and Salette (1987) proposed the concept of the nitrogen nutrition index (*NNI*), which can be expressed by Eq. 4.

$$NNI=N_t/N_c \qquad \text{(Eq. 4)}$$

Where *NNI* is nitrogen nutrition index; N_t is measured value of aboveground biomass nitrogen concentration, g/100 g.

Data analysis

The data were collated and analyzed using Microsoft Excel 2007 and DPS software, and plotted using Origin Pro 8.5 software. In order to evaluate the accuracy of the model, the coefficient of determination (R^2), the mean absolute error (MAE) and the standard mean square error (RMSE) between the simulated and calculated values of the model were calculated (Nash and Sutcliffe 1970; Willmott and Matsuura 2005; Wu *et al.* 2008).

Results

The above-ground biomass of tomato gradually increased with time, while the nitrogen content of tomato plants gradually decreased with time, until end of tomato growth period (DAT=150 d) (Table 1). With full irrigation treatment (W_1), the above-ground biomass and nitrogen content of the plant increased significantly with the increase of nitrogen application rate; compared with full irrigation treatment, only the seedling stage water deficit (W_2) had no significant effect. The above-ground biomass and nitrogen content of the plants increased with an increasing number of days of loss, and the decrease of above-ground biomass and nitrogen content of the plant became larger gradually. The amount and plant nitrogen content were eventually reduced to a minimum, indicating that when sufficient irrigation is carried out, the increasing nitrogen application rate significantly increased the above-ground dry matter and nitrogen content of the plant. On the other hand, when irrigation reduced to 50% during different growth period, the increasing nitrogen application rate can increase biomass to a certain amount (150 kg/hm^2) and it continues to increase along with the nitrogen application rate.

The above-ground biomass and corresponding nitrogen concentrations of plants with different nitrogen and irrigation conditions were calculated to obtain the critical nitrogen concentration on each sampling day. Based on the above-ground biomass and the corresponding critical nitrogen concentrations, a critical nitrogen dilution curve of tomato plants was established (Fig. 1). The coefficients of determination (R^2) for the different water treatments ranged from 0.93 to 0.99, and the fitting degree was extremely significant ($P < 0.01$). Therefore, the model can better reflect the relationship between critical nitrogen concentration and above-ground biomass.

Nitrogen Nutrition Index (*NNI*), as the ratio of nitrogen content to critical nitrogen content of the actual plants, can directly reflect the nutrient status of nitrogen in crops. The *NNI* increases with increasing nitrogen application rate, and the values range from 0.62–1.21 (Fig. 2). The N_{150} treatments have *NNI* values of less than 1 during the whole growth period, which indicates that the nitrogen demand of the plants is not being met when full irrigation during the whole growth period and reducing irrigation of 50% during the seedling stage. At $N_{300,}$ *NNI* is around 1, and when fully watered, the only optimum nitrogen application rate at the seedling stage was N_{300}. The *NNI* of the N_0 treatments during the whole growth period are less than 1 when reducing irrigation of 50% during the seedling and flowering stage, during the whole growth period. The *NNI* of N_{150} and N_{300} were greater than 1 after 90 days of growth, and the *NNI* of the N_{150} treatment was closer to 1, indicating that the N_0 and N_{300} treatments were insufficient due to nitrogen deficiency during continuous flowering and dehydration during the whole growth period. Too much nitrogen will inhibit the growth of the plant hence the optimum nitrogen application rate is N_{150}.

The SPAD values of different leaf positions of tomato plants under different water and nitrogen treatments indicated first increasing trend and then decreasing with the number of days after planting (Table 2). At DAT=110 d, the SPAD values of different leaf positions reached the maximum, ranging from 43.4 to 70.6. With the full irrigation treatment (W_1), the values of different leaf positions varied from 21.1 to 70.6, and the SPAD values increased significantly with the increase of nitrogen application rate. The SPAD values of different leaf positions increased significantly with the increase of nitrogen application rate during the deficit period, and also between the N_{150} and N_{300} treatments. Compared with full irrigation during the whole growth period, the SPAD value of different leaf positions of tomato plants were not significantly affected after re-hydration only reducing irrigation of 50% during the seedling stage. The SPAD values of different leaf positions under 50% reduced irrigation during seedling and flowering stages were significantly lower than full irrigation; and when irrigation reduced to 50% during the whole growth period, the SPAD values of different leaf positions in different growth stages were significantly lower than at full irrigation treatments. These findings indicate that the increase of nitrogen application has a significant effect on increasing the SPAD values of different leaf positions. When the irrigation is not sufficient, the nitrogen application rate is effective but only when increased to a certain amount (150 kg/hm^2), and the nitrogen nutrition level is not significantly increased when the nitrogen application is continued. When the nitrogen application rate was held constant, the SPAD values of different leaf positions were significantly higher than deficit treatments, indicating that increasing the irrigation amount can increase the SPAD value of tomato plants, and the SPAD values of the middle leaf in each treatment are higher than in the upper position.

The correlation between the SPAD values of different leaf positions (upper, middle and lower) and the corresponding *NNI* from 2016 to 2018 are given in Fig. 3. The coefficients of determination (R^2) between the SPAD values and the *NNI* of the upper and lower leaves of different water and nitrogen treatments are 0.05–0.93, and the regression relationships between the SPAD values of the upper and lower leaves and *NNI* are affected by different treatments. At DAT=90 d, there is no significant linear relationship between SPAD values and *NNI* except for treatment of reducing irrigation of 50% during seedling and flowering stage; while a good correlation between middle leaf SPAD values and *NNI*, and the coefficients of determination (R^2) were found 0.70–0.98 except for reducing irrigation to 50% during seedling and flowering

Table 1: Effects of water and nitrogen treatments on dynamic accumulation of tomato aboveground biomass in 2016-2018

	Year	DAT/d	Full irrigation for whole crop growth period		Deficit irrigation (50%) at seedling stage				Deficit irrigation (50%) at seedling+ flowering stage			Deficit irrigation (50%) for whole crop growth period		
			N_0	N_{150}	N_{300}	N_0	N_{150}	N_{300}	N_0	N_{150}	N_{300}	N_0	N_{150}	N_{300}
Accumulation of aboveground biomass/10^3 (kg·hm^2)	2016−2017	30	0.56c	0.68b	0.76a	0.47d	0.57c	0.59c	0.56d	0.54c	0.58c	0.48d	0.55c	0.57c
		50	1.05c	1.26b	1.56a	0.87d	1.12c	1.18c	0.87d	1.05c	1.26b	0.89d	1.02c	1.08c
		70	2.36c	2.45b	2.76a	2.15d	2.33c	2.52b	1.79e	2.22d	2.20d	1.83e	2.21d	2.23d
		90	4.65c	5.72b	6.17a	4.42d	5.45b	5.85b	3.68e	4.36d	4.55d	3.63e	4.26d	4.38d
		110	5.82c	6.77b	7.37a	5.68c	7.00b	7.45a	4.7e	5.32d	5.43d	4.75e	5.40d	5.56d
		130	7.31c	7.88b	8.26a	7.41c	8.10b	8.31a	6.41d	6.94d	7.24c	6.12e	6.54d	6.77d
		150	8.87c	10.59b	10.9a	9.02c	10.73b	10.84a	8.78c	9.06c	9.29c	7.38e	7.77d	8.04d
	2017−2018	32	0.58c	0.67b	0.85a	0.51d	0.55c	0.58c	0.49d	0.53d	0.59c	0.51d	0.59c	0.61c
		51	1.18c	1.32b	1.58a	0.91d	1.10c	1.16c	0.89d	1.04d	1.18c	0.94d	1.16c	1.16c
		70	2.49c	2.64b	2.88a	2.17d	2.47c	2.58b	1.73e	2.22d	2.22d	1.92e	2.27d	2.33d
		91	4.87c	5.92b	6.42a	4.49d	5.77b	6.3a	3.65e	4.31d	4.51d	3.90e	4.56d	4.58d
		112	5.94c	7.17b	7.56a	5.63c	7.15b	7.52a	4.80e	5.34d	5.88c	4.98d	5.52d	6.04d
		132	7.62c	7.99b	8.70a	7.22c	8.27b	8.60a	6.46e	7.02d	7.07d	6.35e	6.81d	7.07d
		150	9.13c	10.81b	11.14a	8.83c	10.46b	11.11a	8.67d	9.19c	9.48c	7.60e	8.08d	8.15d
Nitrogen concentration of plant/g·(100g)$^{-1}$	2016-2017	30	2.81d	3.24b	3.50a	2.68e	3.09c	3.18b	2.69e	3.10c	3.21b	2.61e	3.02c	3.25b
		50	2.56d	2.89b	3.22a	2.37e	2.79c	2.87b	2.41e	2.75c	2.92b	2.37e	2.72c	2.86b
		70	2.29c	2.46b	2.61a	2.29c	2.36b	2.58a	2.14d	2.24c	2.37b	2.15d	2.21c	2.39b
		90	1.81d	1.92b	2.15a	1.77d	1.93b	2.16a	1.60e	1.89c	2.04b	1.61e	1.90c	2.03b
		110	1.59d	1.80c	2.06a	1.62d	1.79c	1.98a	1.59e	1.89c	1.92b	1.59e	1.80c	1.93b
		130	1.57d	1.73c	2.05a	1.57d	1.71c	2.01a	1.50e	1.65d	1.53e	1.49e	1.67d	1.90b
		150	1.59d	1.68c	2.00a	1.58d	1.68c	1.93a	1.50e	1.64d	1.86b	1.43e	1.61d	1.67c
	2017−2018	32	2.90d	3.30b	3.50a	2.69e	3.17c	3.32b	2.73e	3.12c	3.32b	2.71e	3.17c	3.32b
		51	2.60d	2.94b	3.17a	2.48e	2.75c	2.97b	2.50e	2.76c	2.90b	2.48e	2.82c	2.89b
		70	2.22d	2.44b	2.60a	2.29c	2.42b	2.56a	2.16d	2.32c	2.41b	2.21d	2.34c	2.43b
		91	1.76d	1.96b	2.15a	1.74d	1.96b	2.22a	1.68e	1.90c	2.06b	1.73e	1.93c	2.08b
		112	1.63d	1.96c	2.15a	1.67d	1.81c	2.09a	1.59e	1.90c	2.00b	1.62e	1.91c	1.95b
		132	1.61d	1.83c	2.05a	1.63d	1.76c	1.98a	1.50e	1.62d	1.91b	1.54e	1.75d	1.84c
		150	1.60d	1.68c	1.95a	1.58d	1.68c	2.00a	1.49e	1.58d	1.90b	1.53e	1.59d	1.74c

Note: W_1-W_4 are full irrigation for the whole crop growth period, deficit irrigation (50%) at seedling stage, seedling + flowering stage, and the whole crop growth period; N_0, N_{150} and N_{300} are nitrogen application rate of 0, 150, 300 kg/hm^2; Data in the table represent average values and those with the same letters are not significantly different ($P < 0.05$), same as below

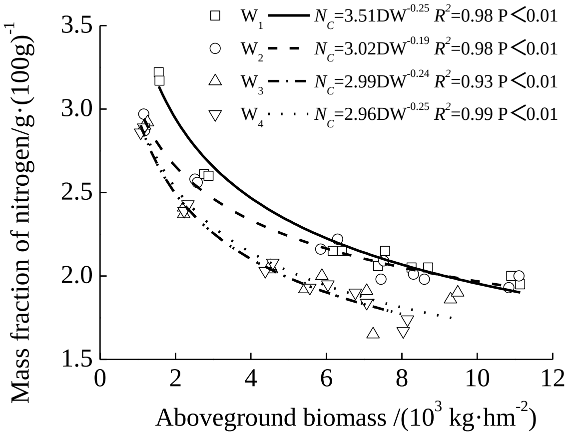


Fig. 1: Construction of nitrogen concentration dilution curves of tomato under different water treatments

Note: W_1-W_4 are full irrigation for the whole growth period, reducing irrigation of 50% during seedling stage, seedling and flowering stage, and the whole growth period, respectively; same as below

stages. At DAT=30 d, in contrast to the treatment of reducing irrigation of 50% during the whole growth stage, all others reached significant levels, indicating a significant positive correlation between SPAD values and *NNI*, *NNI* increased with SPAD, and the fitting relationship was stable. Therefore, *NNI* can be estimated by using the middle leaf SPAD values in different treatments. This approach combines the advantages of SPAD value monitoring with convenience, speed and high *NNI* prediction accuracy for estimating the nitrogen content of tomato plants more quickly and accurately.

The *NNI* and middle leaf SPAD values of different water treatments from 2016 to 2018 were fitted (Fig. 3), and the correlations between different *NNI* and median leaf

Table 2: SPAD values of different leaf positions of tomato under different water and nitrogen treatments in 2016–2018

Leaf position	Year	DAT/d	W_1			W_2			W_3			W_4		
			N_0	N_{150}	N_{300}	N_0	N_{150}	N_{300}	N_0	N_{150}	N_{300}	N_0	N_{150}	N_{300}
Upper leaf	2016-2017	30	24.1c	27.0b	29.3a	23.7c	24.3c	25.1c	23.8c	24.1c	24.3c	23.3c	23.9c	24.5c
		50	32.9c	35.8b	37.9a	29.2d	32.5c	31.6c	29.4d	31.9c	30.6d	30.0d	31.9c	31.7c
		70	40.8c	44.0b	47.0a	35.4d	39.2c	40.9c	34.0d	40.1c	40.1c	38.9d	41.7c	41.7c
		90	50.8c	56.6b	60.1a	47.2d	53.1c	56.6b	42.3e	49.8c	50.5c	45.1e	50.5c	51.8c
		110	55.1c	60.1b	62.9a	52.2c	59.3b	61.7a	48.2d	54.2c	57.1c	49.5d	56.0c	56.3c
		130	49.0c	56.8b	60.8a	50.0c	54.7b	59.7a	47.5d	53.6b	55.4b	37.0e	48.3c	48.8c
		150	41.9c	47.6b	50.5a	42.1c	47.1b	52.0a	41.1c	40.5c	41.9c	36.8d	37.7d	39.0d
	2017-2018	32	23.9c	27.0b	28.9a	23.5c	24.9c	24.9c	23.0c	24.3c	23.8c	23.2c	23.6c	24.1c
		51	32.6c	35.7b	37.2a	29.5d	32.4c	31.5c	29.1d	31.8c	31.6c	30.1d	31.6c	31.4c
		70	40.0c	44.4b	47.0a	35.5d	39.8c	41.4c	34.5d	39.9c	39.5c	36.9d	41.7c	41.7c
		91	50.3c	56.6b	59.0a	46.7d	53.1c	56.3b	42.4e	49.5c	51.4c	44.9e	50.0c	51.3c
		112	53.7c	60.3b	62.5a	52.8c	59.6b	63.1a	48.3d	55.1c	56.9c	48.8d	55.0c	56.9c
		132	47.9c	57.5b	60.1a	49.0c	56.2b	59.7a	45.6d	54.8b	56.4b	36.4e	48.2c	48.8c
		150	42.1c	47.5b	50.8a	43.1c	47.9b	50.7a	40.2c	40.8c	41.4c	36.6d	38.2d	38.5d
Median leaf	2016-2017	30	26.4c	29.8b	32.7a	26.6c	26.1c	27.9c	25.7c	25.7c	27.3c	26.1c	26.0c	27.2c
		50	36.1c	39.3b	42.2a	33.8d	35.5c	35.5c	33.1d	35.2c	35.5c	33.1d	35.0c	35.1c
		70	45.8c	48.9b	52.7a	40.2d	43.2d	48.6b	43.0d	47.0c	46.1c	43.3d	46.3c	46.7c
		90	56.7c	62.9b	67.0a	52.8d	57.8c	64.7b	49.7e	56.0c	56.6c	50.5e	55.9c	57.4c
		110	61.1c	67.2b	69.9a	61.3c	68.4b	68.8a	55.7d	60.8c	64.0c	55.5d	62.3c	64.4c
		130	59.0c	66.5b	69.6a	60.4c	66.2b	68.5a	54.2d	58.3c	60.5c	46.5e	55.3d	56.6d
		150	47.5c	57.3b	61.2a	48.4c	57.9b	62.0a	43.9d	44.4d	45.4d	40.6e	42.2e	42.5e
	2017-2018	32	26.6c	29.4b	32.5a	26.2c	26.3c	27.2c	25.8c	26.2c	28.1c	25.7c	25.9c	27.5c
		51	36.1c	39.2b	41.3a	34.4d	36.0c	35.8c	33.8d	35.9c	35.8d	32.8d	35.6c	35.7c
		70	45.2c	48.8b	52.3a	40.7d	43.4d	47.9b	43.0d	46.2c	46.1c	42.9d	46.5c	45.5c
		91	57.1c	62.9b	66.9a	52.6d	58.2c	64.0b	50.4e	57.1c	57.7c	49.9e	55.0c	56.5c
		112	60.2c	67.8b	70.6a	61.3c	67.4b	70.6a	54.6d	61.4c	64.5c	55.5d	61.2c	63.1c
		132	58.2c	67.0b	69.0a	60.6c	67.4b	69.5a	54.4d	59.3c	61.9c	45.8e	54.3d	56.4d
		150	46.8c	57.4b	60.8a	47.7c	59.6b	60.6a	43.7d	45.0d	45.6d	39.9e	40.5e	41.0e
Lower leaf	2016-2017	30	21.7c	23.7b	26.3a	21.0c	21.5c	21.8c	20.8c	21.4c	21.7c	21.1c	20.8c	21.8c
		50	28.6c	31.5b	33.5a	26.2d	27.8c	29.0c	26.5d	28.4c	28.9c	26.6d	27.9c	28.2c
		70	36.3c	39.2b	42.1a	31.9d	34.5d	38.5b	34.3d	37.9c	36.8c	34.6d	37.2c	37.3c
		90	46.0c	50.3b	54.1a	40.6d	46.7c	50.8b	40.5d	46.1c	46.8c	41.0d	45.2c	45.9c
		110	48.9c	53.4b	56.4a	50.2c	54.2b	57.6a	43.4d	50.2c	51.6c	44.1d	49.0c	51.3c
		130	38.4c	45.9b	48.3a	38.6c	46.1b	47.5a	38.0c	44.4b	47.0b	32.7d	38.3c	38.4c
		150	37.1c	42.5b	45.8a	36.7c	42.7b	45.5a	35.3d	35.6d	34.9d	26.6e	26.7e	27.8e
	2017-2018	32	21.1c	23.2b	26.4a	21.4c	21.8c	22.0c	20.6c	21.9c	21.5c	20.8c	20.5c	21.6c
		51	28.7c	31.4b	34.0a	26.3d	27.0c	29.6c	26.6d	28.6c	29.5c	26.4d	27.5c	27.7c
		70	36.4c	39.7b	41.7a	32.3d	33.9d	39.5b	34.3d	38.8c	37.8c	34.4d	36.6c	37.5c
		91	45.2c	49.7b	53.7a	41.1d	47.3c	49.8b	41.7d	44.4c	47.3c	40.0d	43.6c	46.6c
		112	47.8c	52.8b	56.5a	50.2c	53.5b	58.4a	43.2d	49.7c	52.0b	43.4d	49.5c	50.5c
		132	37.7c	45.6b	47.8a	38.3c	46.2b	47.4a	38.3c	45.0b	44.1b	32.2d	38.2c	37.9c
		150	36.5c	42.8b	45.0a	36.2c	44.0b	45.4a	30.1d	36.3c	37.3c	26.9d	29.0d	27.8d

SPAD values were different. The equation of its fitting is:

$$NNI = k \cdot \text{SPAD} + m \qquad \text{(Eq. 5)}$$

Where *NNI* is the nitrogen nutrition index of different treatments; SPAD is the SPAD values of different treatments' middle leaves; *k* and *m* are the fitting equation parameters, and the *k* and *m* values obtained by different water treatment fittings are shown in Fig. 4.

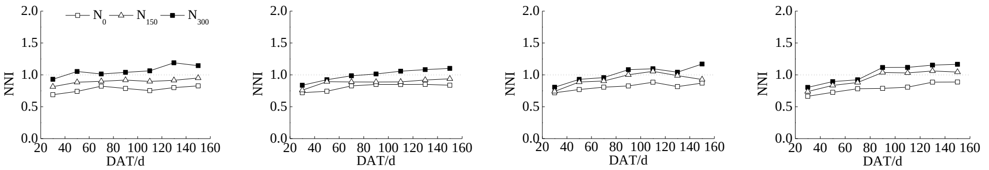
The relationships between the SPAD and *NNI* of tomato plants in different water treatments were different. Therefore, using the data from 2016 to 2018, the average daily water consumption of tomatoes in different water treatment days were fitted with the parameters *k* and *m*. The fitting relationship between *m* and tomato daily water consumption (Table 3) indicated that the fitting relationship parameters *k* and *m* between the tomato leaf position SPAD values and *NNI* have a significant relationship with the daily water consumption of the corresponding tomato plants (Fig. 4). Therefore, the parameters *k* and *m* can be obtained from different daily water consumption levels of tomato. Given the linear relationship between the middle leaf SPAD and *NNI*, the tomato plant *NNI* can be estimated based on the tomato plant mid-valence SPAD value for nitrogen nutrition diagnosis. Using *NNI*=1 as the standard for appropriate nitrogen application, *NNI*>1 or *NNI*<1 indicate that nitrogen is applied either excessively or insufficiently. Therefore, this method is used to simulate *NNI* with different water treatments, which further provides guidance for nitrogen nutrition diagnosis.

Discussion

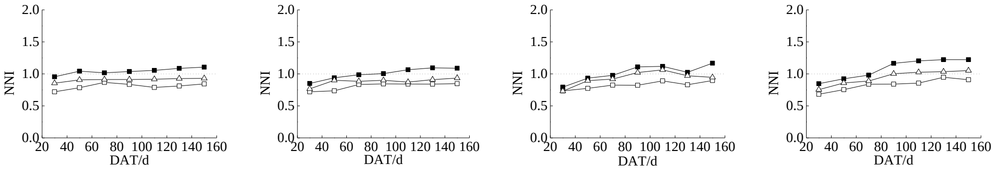
Water and nitrogen are important factors affecting plant growth. Different water and nitrogen treatments affected crop development and dry matter accumulation in tomato plants under greenhouse, which in turn affected nitrogen uptake. Yang *et al.* (2015) in a study on tomato showed that the critical nitrogen application rate was different under

Table 3: Daily water consumption of greenhouse tomato under different treatments in 2016-2018 mm·d^{-1}

DAT/d	2016-2017				2017-2018			
	W_1	W_2	W_3	W_4	W_1	W_2	W_3	W_4
30	1.3a	0.9b	1.1b	1.0b	1.4a	0.9b	1.0b	0.9b
50	1.8a	1.3b	1.5b	1.5b	1.7a	1.2b	1.3b	1.4b
70	2.1a	1.9b	1.7c	1.7c	2.2a	1.9b	1.6c	1.7c
90	2.3a	2.2a	1.8b	1.7b	2.2a	2.1a	1.5b	1.5b
110	2.1a	2.2a	1.2b	1.2b	1.9a	1.9a	1.2b	1.3b
130	1.2a	1.2a	1.1b	0.7c	1.0a	1.0a	0.9b	0.7c
150	0.7a	0.6a	0.6a	0.4b	0.5a	0.5a	0.4a	0.4b
mean	1.6a	1.4a	1.3b	1.2b	1.6a	1.4a	1.2b	1.2b



a. 2016—2017, W_1 b. 2016—2017, W_2 c. 2016—2017, W_3 d. 2016—2017, W_4

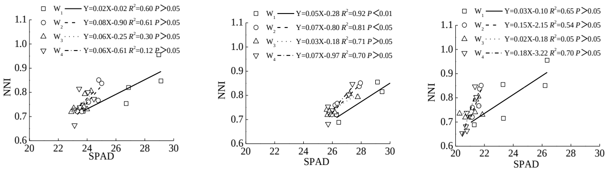


e . 2017—2018, W_1 f. 2017—2018, W_2 g. 2017—2018, W_3 h. 2017—2018, W_4

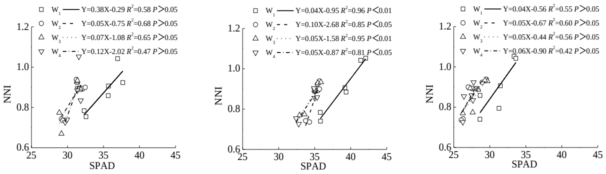
Fig. 2: Dynamic change of nitrogen nutrition index (*NNI*) of tomato under different water and nitrogen treatments in 2016–2018

different water regimes. The critical nitrogen concentration of plants under high water treatment was larger, and irrigation could promote the nitrogen uptake of plants. This study also showed that increasing the amount of irrigation and nitrogen application could increase the critical nitrogen concentration of tomato plants, which can be obtained by critical nitrogen dilution curve parameters *a* and *b* for different water treatments by fitting the curve. Various factors have different effects on parameters *a* and *b* of the critical nitrogen dilution curve. Zhao *et al.* (2012) showed that parameter *a* and *b* the protein content of the varieties were positively correlated due to the differential abilities of different plants to absorb and assimilate nitrogen. Xiang *et al.* (2016) studied the applicability of the nitrogen concentration dilution model in greenhouse peppers, and obtained the critical nitrogen dilution curve parameter *a*; and found that the irrigation amount first increased and then decreased, while parameter *b* did not change significantly with the change of irrigation amount.

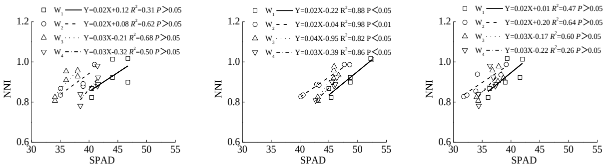
The SPAD-chlorophyll meter has the advantages of easy portability and providing real-time measurements in the field, and widely used to monitor the nitrogen application levels of various crops such as cotton (Wu *et al.* 1998), wheat (Debaeke *et al.* 2006) and corn (Singh *et al.* 2011). The correlation between leaf SPAD values and *NNI* has been reported in maize (Ziadi *et al.* 2008) and wheat (Prost and Jeuffroy 2007), and these studies presented relative SPAD values and *NNI* for maize and wheat leaves. Debaeke *et al.* (2006) showed non-linear relationship between wheat leaf relative SPAD values and *NNI* that was not significantly affected by the year, variety or growth period. Yang *et al.* (2014) showed that the SPAD values of different leaf positions and *NNI* showed different linear relationships, and the stability of different leaf positions varied. In present studies (Ziadi *et al.* 2008; Yang *et al.* 2014), when the leaf SPAD value and *NNI* fitting degree of a leaf position were higher, then the stability was higher. Therefore, it can be used to determine the ideal leaf position for the diagnosis of nitrogen. This study also showed a linear correlation between SPAD and *NNI* in leaves of tomato plants with different water treatments. The degrees of fitting between SPAD values and *NNI* of upper and lower leaves were poor and affected by year and treatment. With a significant positive linear correlation between the SPAD values of the middle leaf and *NNI*, and good stability, the tomato middle leaf is suitable for nitrogen diagnosis. Moreover, the fitting parameters between tomato leaf SPAD values and *NNI* were significantly correlated in non-linear fashion with the average water consumption of tomato.



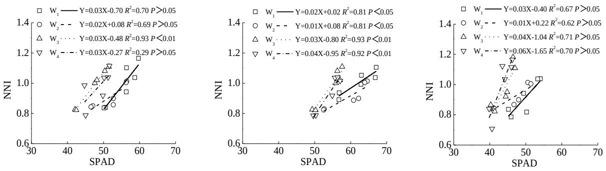
a.DAT=30 d, Upper leaf b.DAT=30 d, Median leaf c.DAT=30 d, Lower leaf



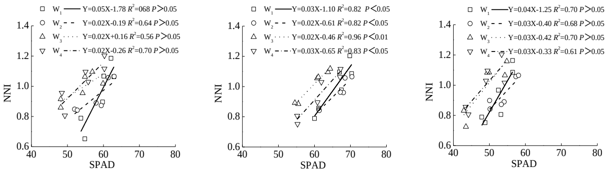
d.DAT=50 d, Upper leaf e.DAT=50 d, Median leaf f.DAT=50 d, Lower leaf



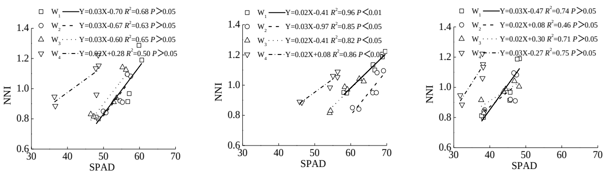
g.DAT=70 d, Upper leaf h.DAT=70 d, Median leaf i.DAT=70 d, Lower leaf



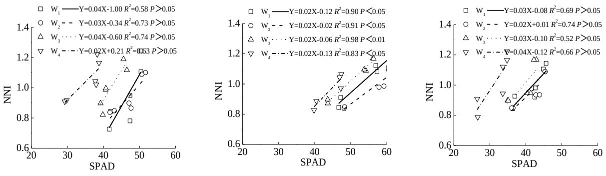
j.DAT=90 d, Upper leaf k.DAT=90 d, Median leaf l.DAT=90 d, Lower leaf



m.DAT=110 d, Upper leaf n.DAT=110 d, Median leaf o.DAT=110 d, Lower leaf



p.DAT=130 d, Upper leaf q.DAT=130 d, Median leaf r.DAT=130 d, Lower leaf



s.DAT=150 d, Upper leaf t.DAT=150 d, Median leaf u.DAT=150 d, Lower leaf

Fig. 3: Relationship between SPAD and NNI of different tomato leaf positions in 2016–2018

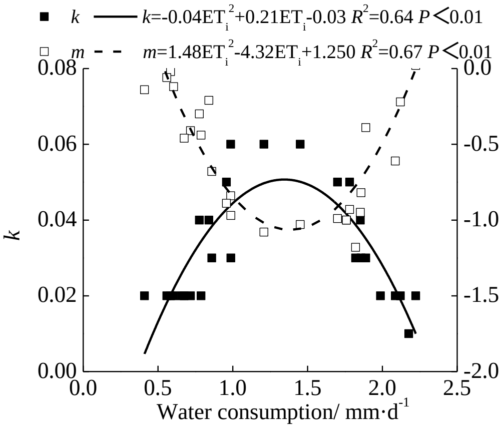


Fig. 4: Relationship between Daily tomato water consumption and coefficient k and m

Note: m and k are empirical coefficients of different water treatments; ET_i is average daily water consumption of tomato within i days under different water treatments, respectively

Therefore, a linear relationship between SPAD values and *NNI* of the tomato plant middle leaf was obtained by different water treatment or daily water consumption levels. The *NNI* values of one (1) was used as the ideal nitrogen nutrition status index value, and the "optimal" SPAD value at *NNI*=1 was obtained according to the different water consumption levels of tomato plants.

This optimal SPAD value can then be used as the appropriate value for nitrogen nutrition diagnosis. For example, when the SPAD value of the middle leaf in the test treatment is larger than the optimal SPAD value, it indicates that the excessive application of nitrogen should be reduced. Conversely, when the SPAD value of the middle leaf in the test treatment is less than the optimal SPAD value, it indicates that the application of nitrogen should be increased. Therefore, it is possible to accurately determine the nitrogen nutrition status by comparing the different treatment SPAD values with the optimal SPAD values and adjust the nitrogen application rate appropriately in real-time.

Conclusion

The results showed that relationship between the critical nitrogen concentration of tomato and the maximum above-ground biomass is $N_C=a\cdot DW^{-b}$, where parameter *a* is 2.96–3.51, parameter *b* is 0.19–0.25. The critical nitrogen concentration of plants under different water treatments can be accurately estimated. The use of SPAD values of different leaves for tomato nitrogen nutrition diagnosis showed that the ideal leaf position was the middle leaf. There is a good linear relationship between the SPAD values of tomato leaves and the nitrogen nutrient index *NNI* ($NNI=k\cdot SPAD+m$), and parameters *k* and *m* have significant nonlinear correlations with the daily water consumption of tomato plants. The daily water consumption was estimated by parameters *k* and *m*, and then the SPAD values on different days after planting for *NNI*=1 were simulated. The SPAD value was used as a criterion for judging the nutritional status of nitrogen in order to accurately diagnose the nitrogen nutrition status of the plants.

Acknowledgments

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